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Minireview

Cellular prion protein: on the road for functions

Vilma R. Martins[a,*], Rafael Linden[b], Marco A.M. Prado[c], Roger Walz[d], Américo C. Sakamoto[d], Ivan Izquierdo[e], Ricardo R. Brentani[f]

[a] *Centro de Tratamento e Pesquisa Hospital do Câncer, São Paulo, SP, Brazil*
[b] *Laboratório de Neurogênese, Instituto de Biofísica da UFRJ, Rio de Janeiro, Brazil*
[c] *Laboratório de Neurofarmacologia, Departamento de Farmacologia, ICB, UFMG, Belo Horizonte, Brazil*
[d] *CIREP, Centro de Cirurgia de Epilepsia, Faculdade de Medicina de Ribeirão Preto, USP, Ribeirão Preto, SP, Brazil*
[e] *Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Porto Alegre, RS, Brazil*
[f] *Ludwig Institute for Cancer Research, São Paulo Branch, São Paulo, SP, Brazil*

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Abstract Cellular prion (PrPc) is a plasma membrane glycosyphosphatidylinositol-anchored protein present in neurons but also in other cell types. Protein conservation among species suggests that PrPc may have important physiological roles. Cellular and molecular approaches have established several novel features of the regulation of PrPc expression, cellular trafficking as well as its participation in copper uptake, protection against oxidative stress, cell adhesion, differentiation, signaling and cell survival. It is therefore likely that PrPc plays pleiotropic roles in neuronal and non-neuronal cells, and as such the loss of function of PrPc may be an important component of various diseases.

Key words: Cellular prion protein; Gene regulation; Trafficking; Laminin; Programmed cell death; Human diseases

1. Introduction

The cellular prion protein (PrPc) was identified not because of its cellular function but rather due to its involvement in neurodegenerative diseases called spongiform encephalopathies [1]. Based on previous approaches to viral oncogenes, scientists were looking for an organism that coded a new type of protein responsible for cellular changes. The surprise was that, in fact, the molecule identified was coded by the cellular genome.

It is believed that the infectious particles, responsible for the transmissible forms of prion diseases are composed by a single protein, that is a PrPc isoform with only a distinct secondary structure. Therefore, it was proposed that infectivity is a consequence of conformational modification of PrPc by the infectious protein prion scrapie (PrPsc) [1]. Due to the uncommon characteristics of these diseases, efforts were mainly directed at understanding the infection, while the physiological roles of PrPc were mostly ignored.

Herein we report several aspects of PrPc gene expression, regulation and trafficking. We also discuss interactions between PrPc and the extracellular matrix glycoprotein laminin [2,3] as well as properties of a peptide predicted to mimic the docking site of a putative PrPc membrane ligand (p66) [4]. Such interactions elicit signal transduction via PrPc, which can lead both to neuroprotection (Chiarini et al., J. Biol. Chem., in press) and neuronal differentiation [2,3]. In addition, the role of copper binding [5] in the internalization of PrPc [6,7] is discussed.

2. PrPc expression regulation

Prn-p (the gene that codes for PrPc) contains three exons in mouse and rat and two exons in hamster and humans, with the third and second exons, respectively, encoding the entire protein of approximately 250 amino acids. Two signal peptides are present in the molecule, one at the N-terminus, which is cleaved during the biosynthesis of PrPc in the rough endoplasmic reticulum and the second at the C-terminus, that allows for attachment to a glycosyphosphatidylinositol (GPI) anchor. Two glycosylation sites are also mapped to the C-terminus (reviewed in [1]).

It was believed that Prn-p is a housekeeping gene, since its mRNA is constitutively expressed in tissues from adult animals [8]. However, PrPc expression is highly regulated both during development [9] and by nerve growth factor (NGF) [2]. Regulation of PrPc levels is associated both with a physiological function [2] and with the rate of prion infection [10].

The Prn-p gene promoter has been identified in mouse [11], hamster [12], rat [13], cattle [14] and human [15], and the major region of transcriptional control was found upstream of the initiation site. However, other regulatory regions were also identified [11,14]. All reported Prn-p promoters lack a TATA box and contain GC-rich features, which are potential binding sites for Sp-1 transcriptional factors. Specific sites for AP-1 and AP-2 were also identified [11,13,15]. However, it is believed that these ubiquitous transcription factors cannot account for tissue specific regulation in adult animals [8].

*Corresponding author. Fax: (55)-11-3207 7001.
E-mail address: vmartins@ludwig.org.br (V.R. Martins).

Abbreviations: GFP, green fluorescent protein; GPI, glycosyphosphatidylinositol; NGF, nerve growth factor; p66, putative PrPc membrane ligand; Prn-p, gene that codes for the cellular prion protein; PrPc, cellular prion protein; PrPsc, prion scrapie, infectious protein

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It is tempting to speculate that Prn-p promoter activity may be controlled by tissue specific transcription factors, but either promoter methylation or changes in chromatin structure may also occur. It is important to note that transcriptional repression can be mediated by histone deacetylase 1 targeting to Sp-1 [16], whose binding element is present in all described Prn-p promoters. In fact, we have observed that Prn-p promoter activity is highly dependent on chromatin structure (Cabral et al., J. Biol. Chem., in press), indicating that PrPc expression may be regulated.

3. Cellular traffic of PrPc

Internalization of PrPc from the plasma membrane occurs constitutively in cultured cells and a large part of the protein recycles back to the plasmalemma without degradation [17]. Initial experiments have shown the presence of PrPc in endocytic organelles [17] and later the involvement of clathrin-coated vesicles was proposed [18]. However, PrPc is present in rafts at the plasma membrane where most GPI-anchored proteins appear to accumulate [19] and may undergo caveolar-like mechanism of internalization. Since PrPc lacks transmembrane domains to assist interactions with clathrin adapter proteins, it has been proposed that PrPc internalization does not depend on clathrin-mediated endocytosis [20] or that ancillary proteins are required [4,18].

In order to visualize PrPc traffic, we [7] and others [21] have tagged mouse or bovine PrPc with green fluorescent protein (GFP). The GPI-anchored fluorescent fusion protein appears to be functional [7,21]. One of the hallmarks of PrPc traffic is its copper-induced internalization [6,22]. We followed GFP-PrPc traffic in living neuronal cells and found that the fluorescent protein is internalized from the plasma membrane and accumulates at a perinuclear region in response to copper [7]. The localization of internalized GFP-PrPc is still under investigation, however one of the compartments labeled by the fluorescent protein at steady state is the Golgi apparatus [21]. We have also observed that part of the perinuclear labeling is co-localized with internalized transferrin, FM4-64 and a Rab-5 mutant, all of which are markers of classical endocytic organelles [23]. PrPc internalization mediated by copper depends on the originally described N-terminal octarepeat domain [5] rather than on the newly described copper-binding motif [24]. This methodology may provide novel information on the physiological pathways followed by PrPc inside cells.

4. PrPc physiological function unsolved by generation of PrPc null mice

In 1992 Charles Weissmann's group [25] generated PrPc-null mice and reported that PrPc expression is dispensable for normal development and behavior. In agreement with these results, we further demonstrated [26] that such animals had normal performance in short- and long-term fear-motivated memory, anxiety and exploratory behavior. Moreover, alterations both in circadian activity and sleep were also described [27].

The role of PrPc is still unclear even after the generation of three additional strains of PrPc null mice. One of them, Npu, showed normal development while the other two, Ngsk and Rcm0 developed late onset ataxia (reviewed in [28]). These apparent contradictory results were solved by cloning a gene located 16 kb downstream from Prn-p gene, called Doppel, which generates two major transcripts of 1.7 and 2.7 kDa, as well as an unusual chimeric transcript generated by intergenic splicing with Prn-p. Interestingly, the chimeric transcript is up-regulated in strains of PrPc null mice that show ataxia, but not in those with a normal phenotype, suggesting that the overexpression of Doppel rather than the absence of PrPc is the cause of neurodegeneration [28].

Taken together, these data suggest that animals without PrPc develop normally and have normal behavior in most of the tests applied. These results may be due to compensatory mechanisms as previously described [25]. However, it may be argued that the right test should be applied to find the altered phenotype.

The generation of conditional PrPc-null mice would be important to solve questions regarding compensatory mechanisms. However, an alternative approach is to acutely block PrPc, for example by the use of specific antibodies, such that protein function is not compensated.

5. In vitro experiments show that PrPc mediates neuronal cell adhesion, differentiation and survival

Various PrPc-binding proteins have been identified, but the role of most of these interactions remains to be established (reviewed in [29]). Strong evidence for PrPc function derives from its interaction with laminin [2,3] because binding of the latter to its classical receptor integrins plays a pivotal role in cell proliferation, differentiation, migration and death [30].

We found that PrPc is a high affinity receptor for laminin, with a binding site at the carboxy-terminal decapeptide (RNIAEIKDI) of the laminin γ-1 chain [2]. This domain had been shown to mediate neurite formation but the nature of its cellular ligand was obscure [31]. Neurite extension elicited by intact laminin was inhibited by anti-PrPc antibodies, while that mediated by the γ-1 chain decapeptide was completely abolished. Furthermore, neurons from PrPc-null mice grew fewer neurites on laminin than wild type animal cells, and no neuritogenesis was induced by the γ-1 chain decapeptide [2]. Finally, we also found that the PrPc–laminin interaction affects both neuronal cell adhesion and neurite maintenance [3].

PrPc is expressed in most tissues and laminin γ-1 chain is the most conserved of all laminin types [30]. Thus, we predict that their interaction may be important not only in the nervous system but in other tissues as well.

PrPc also interacts with the 37-kDa/67-kDa laminin receptor, which may participate in the internalization of 20–50% of the membrane-bound PrPc [32], indicating the need of other components to mediate PrPc internalization. It is interesting to note that both PrPc and laminin interact with the same domain at the laminin receptor, suggesting that laminin receptor–PrPc binding excludes laminin receptor–laminin interaction [33]. On the other hand, the laminin receptor and PrPc bind to distinct domains of laminin, in either the α1 [34] or the γ-1 chains [2], respectively. These alternative associations may be interpreted as combinatorial association events leading to various signal transduction pathways, and their biological relevance needs to be addressed.

Cell culture experiments indicated that PrPc affects neuronal survival under stress. Copper-bound PrPc shows super-

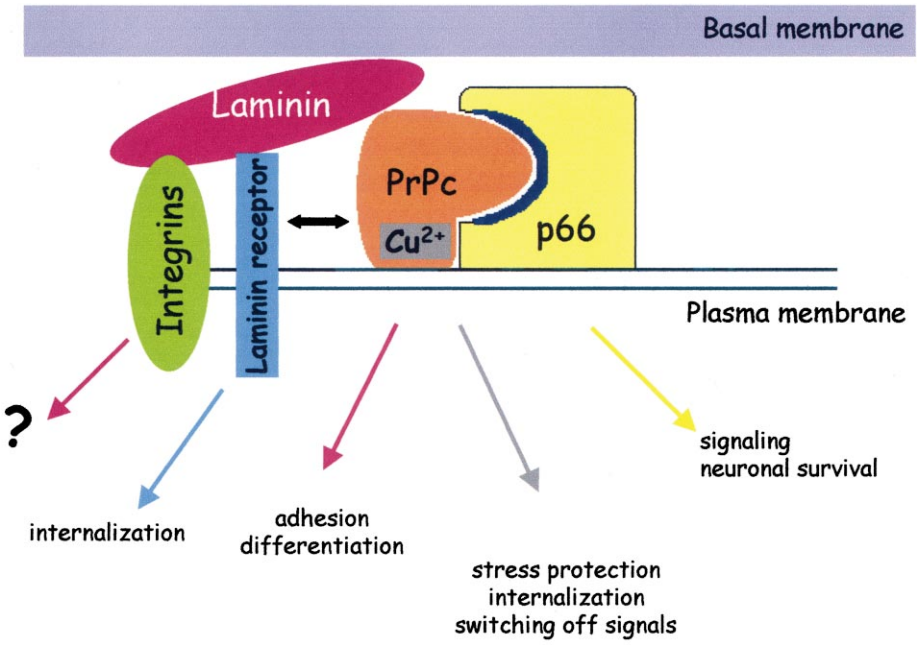

Fig. 1. A multi-functional PrPc-based protein complex. PrPc interaction with laminin mediates neuronal adhesion and differentiation. The binding of a peptide (dark blue) that mimics a PrPc putative ligand, p66, induces cell signaling and promotes cell survival. The complex PrPc–p66 may be formed between proteins either in the same or in distinct cells. Copper uptake by PrPc protects against oxidative stress, induces protein internalization and may be involved with switching off mechanisms. Laminin receptor seems to be involved with protein internalization and the role of integrins is still under investigation.

oxide dismutase activity [35], and PrPc-null cultured neurons are more sensitive than wild-type neurons to oxidative stress in vitro [36]. Thus, the loss of PrPc-mediated anti-oxidant function may be involved in prion diseases [37]. Indeed, serum-deprived PrPc-null mouse cell lines were more sensitive than wild type cells to programmed cell death [38]. In addition, overexpression of PrPc in human primary neurons protected against apoptosis induced by overexpressed Bax [39].

We found that a PrPc-binding peptide, containing sequences found in the putative PrPc p66 ligand previously described [4], partially prevented programmed cell death of undifferentiated post-mitotic retinal cells (Chiarini et al., submitted for publication). The same peptide both increased intracellular cAMP, and activated the Erk pathway in the retina, but the neuroprotective effect required activity of cAMP-dependent protein kinase (PKA) rather than MAP kinase (Chiarini et al., submitted for publication). The mechanism by which PrPc stimulates adenylyl cyclase remains to be investigated, but activation of G-proteins by various GPI-anchored molecules has been shown [40].

These data raise the intriguing possibility that PrPc may be a neurotrophic receptor for p66. In combination with the loss of direct anti-oxidant activity, depletion of PrPc would deprive nervous tissue of intracellular neuroprotective pathways, thereby lowering the threshold for programmed cell death in prion diseases [41].

Antibodies to PrPc lead to caveolin-dependent activation of the Fyn tyrosine kinase in a neuroectodermal cell line, following induction of neuron-like differentiation [42]. Although consistent with signaling through PrPc in membrane lipid rafts, both the artificial conditions and dependence on cross-linking preclude conclusions regarding physiological roles of PrPc.

6. A putative role for PrPc dysfunction in pathologies other than classical prion diseases

With respect to neurological diseases involving excitotoxicity and neuroplasticity, we recently found that PrPc-null mice are more susceptible to acute seizures and kindling induced by various protocols [43]. Since oxidative stress plays a role in pathophysiology both during and after seizures [44], an impairment of brain anti-oxidant defences in PrPc-null mice [45] may play an important role in their lower threshold to seizures. Moreover, these animals showed changes in the activity of ectonucleotidases (apyrase and 5′-nucleotidase) of neocortical and hippocampal synaptosomes, that could result in lower levels of endogenous anti-convulsant adenosine in the synaptic cleft [46].

Regarding human neurological and psychiatric illnesses, a familial form of an atypical psychiatric disorder was associated with a higher prevalence of a PrPc gene mutation [47]. The relationship of these findings with the physiological roles of PrPc remains to be elucidated.

Finally, protein and lipid oxidation in association with lower catalase activity are greatly increased in skeletal muscle, heart, and liver of PrPc-null mice [45]. These data suggest that PrPc may have an important role in oxidative stress not only in the central nervous system but also in other organs. If this is the case, the ‘physiological prion protein system’ may be implicated not only in acute or chronic neurological and psychiatric diseases, but also with other human illnesses, such as myocardial infarction (heart reperfusion injury), hepatic failure and sepsis among others.

7. A PrPc-based multi-functional protein complex

The involvement of PrPc in protection against oxidative

stress, cell adhesion, differentiation, signaling and survival is attributed to its interaction with copper, laminin and p66 (Fig. 1). PrPc is likely a part of a multi-component protein complex, and due to its cellular localization it may connect signals from the extracellular matrix to the intracellular milieu. PrPc interaction with laminin induces cell adhesion and neuritogenesis, and a peptide that mimics PrPc–p66 binding mediates cell survival. The role of copper in the internalization of PrPc can be interpreted either as a mechanism of protection against oxidative insults, or as a mechanism for switching off elicited signals. This complex organization and the contribution of other proteins such as integrins and the laminin receptor [32] in these processes merit further investigation. In conclusion, the evidence for multiple functions of PrPc supports the hypothesis that either depletion or mutations in the cellular prion may lead to loss-of-function components in prion diseases [48].

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